REMARKS

In response to the request for a list of copending applications directed to subject matter similar to the present claims, Applicants offer the following list of applications owned by the assignee of the present application. The claims are provided in the attached appendices.

Serial No.	Title	Appendix with Claims
11/451,472	*Estrogen mixture, recovery from pregnant mare urine (PMU), module for enrighment and stablization*	A
10/857,355	*Estrogen mixture, recovery from pregnant mare urine (PMU) by solid phase extraction by means of RP-silica gel-Intermediate washing*	B
10/681,284 (present application)	*Estrogen mixture, recovery from pregnant mare urine (PMU) by liquid extraction with ethylacetate*	C
10/890,390	*Conjugated estrogen mixture, recovery from pregnant mares' urine (PMU) by solid phase extraction using ethylacetate*	D

If there are any questions regarding this amendment or the application in general, a telephone call to the undersigned would be appreciated since this should expedite the prosecution of the application for all concerned.

If necessary to effect a timely response, this paper should be considered as a petition for an Extension of Time sufficient to effect a timely response, and please charge any deficiency in fees or credit any overpayments to Deposit Account No. 05-1323 (Docket No. 029300.52141US).

Respectfully submitted,

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/Christopher T. McWhinney/
J. D. Evans
Registration No. 26,269

Christopher T. McWhinney
Registration No. 42,875

CROWELL & MORING LLP
Intellectual Property Group
P.O. Box 14300
Washington, DC 20044-4300
Telephone No.: (202) 624-2500
Facsimile No.: (202) 628-8844
JDE:CTM:mdm (3189324)

APPENDIX A

Claims:

1. A method for concentrating and stabilizing mixtures of conjugated estrogens from pregnant mare urine in a decentralized manner that can be carried out in the field, comprising

pumping a predetermined maximum total amount of collected liquid pregnant mare urine from a supply vessel at a predetermined flow rate through an upright cartridge containing a solid adsorbent support capable of adsorbing a predetermined quantity of conjugated estrogens such that the adsorbent is surrounded by a flow of the pumped liquid urine, whereby a natural mixture of conjugated estrogens from the urine is concentrated and stabilized by adsorption an said support;

wherein the maximum total amount of liquid urine pumped through the cartridge is matched to the maximum loading capacity of the adsorbent for conjugated estrogens contained in the liquid urine, and pumping of the liquid urine through the cartridge is ceased at or before maximum loading of the adsorbent with conjugated estrogens is achieved;

discarding liquid urine effluent depleted in conjugated estrogens from the cartridge,

wherein the cartridge is not rinsed with a preservative solution containing a preservative agent after loading of the adsorbent with conjugated estrogens;

storing said upright cartridge containing the solid adsorbent support with the concentrated and stabilized natural mixture of conjugated estrogens adsorbed thereon, and

transporting said upright cartridge containing the solid adsorbent support with the a concentrated and stabilized natural mixture of conjugated estrogens from the urine to a processing site, where the conjugated estrogens are eluted from the adsorbent.

2. A method according to claim 1, wherein the preservative agent is selected from customary preservatives, germicides, bactericides and antihelmintic agents.

3. A method according to claim 1 wherein the liquid urine has been previously pre-purified by coarse mechanical filtration.

4. A method according to claim 1 wherein the urine is pumped continuously through said cartridge.

5. A method according to claim 1 wherein the urine is pumped through said cartridge in discrete portions.

6. A method according to claim 5 wherein the discrete portions of urine are pumped through said upright cartridge over a time period of several days partially or completely overlapping with the storage time period.

7. A method according to claim 1 wherein said cartridge has internal dimensions such that the cartridge can accommodate from 30 to 50 liters of adsorbent, and the pregnant mare urine is required to travel a path distance of 80 to 120 cm through the cartridge.

8. A method according to claim 1 wherein the cartridge is made of impact-resistant laboratory glass, plastic, or metal.

9. A method according to claim 1 wherein the liquid urine is pumped by a monopump, a hose pump, or membrane pump.

10. A method according to claim 1, wherein the flow rate is measured by flow meter selected from the group consisting of rotameter with a float, a vane air flow meter, or an inductive flow meter.

11. A method according to claim 1 wherein the quantity of liquid urine pumped through the cartridge is measured by a throughput meter.

12. A method according to claim 11 wherein said throughput meter is a water meter or an inductive throughput meter.

13. A method according to claim 1 wherein the urine flow has a flow velocity ranging from 3 to 10 adsorber bed volumes per hour.

14. A method according to claim 13 wherein the urine flow velocity ranges from 4 to 6 adsorber bed volumes per hour.

15. A method according to claim 1 wherein the adsorbent is selected from the group consisting of polymeric adsorber resins, silica gel, or RP silica gel.

16. A method according to claim 14 wherein the adsorbent is a polymeric adsorber resin.

17. A method according to claim 14 wherein the polymeric adsorber resin is a semipolar polymeric adsorber resin.

18. A method according to claim 17 wherein the semipolar polymeric adsorber resin has an intermediate polarity having a dipole moment of the active resin surface in a range of 1.0 to 3.0 Debye.

19. A method according to claim 17 wherein the semi-polar polymeric adsorber resin is a macroporous polycarbaxylic acid ester resin.

20. A method according to claim 19 wherein the macroporous polycarboxylic acid ester resin is a cross-linked aliphatic polycarboxylic acid ester resin.

21. A method according to claim 20 wherein the cross-linked aliphatic polycarboxylic acid ester resin has a macroreticular structure.

22. A method according to claim 20 wherein the cross-linked aliphatic polycarboxylic acid ester resin is a cross-linked polyacrylic ester resin.

23. A method according to claim 1 wherein a maximum total quantity of liquid urine of from 20 to 60 adsorber bed volumes is passed through said cartridge.

24. A method according to claim 23 wherein the maximum total quantity of liquid urine is 30 to 40 adsorber bed volumes.

25. A method according to claim 1 wherein after loading of the adsorbent with a quantity of pregnant mare urine, the cartridge is rinsed with an aqueous wash solution.

26. A method according to claim 25 wherein the aqueous wash solution is a basic wash solution.

27. A method according to claim 26 wherein the basic wash solution is a diluted aqueous sodium hydroxide solution.

28. A method according to claim 27 wherein the diluted aqueous sodium hydroxide solution is an aqueous 0.5 -2N sodium hydroxide solution.

29. A method according to claim 25 wherein the rinsing is carried out after the adsorbent has been loaded with a quantity of pregnant mare urine containing an amount of conjugated estrogens corresponding approximately to the total capacity of the adsorbent to adsorb conjugated estrogens.

30. A method according to claim 25 wherein the cartridge is rinsed with water.

31. A method according to claim 25 wherein after loading of the adsorbent with a quantity of pregnant mare urine, the cartridge is rinsed with an aqueous wash solution, and thereafter further stabilized by rinsing with a pH-adjusted aqueous solution or an aqueous salt solution.

32. A method according to claim 31 wherein the cartridge is stabilized by rinsing with an aqueous salt solution.

33. A method according to claim 32 wherein the aqueous salt solution is a sodium chloride solution having a salt concentration of approximately 10 to 35 wt-%.

34. A method according to claim 32 wherein the aqueous salt solution is a sodium chloride solution having a salt concentration of approximately 25 to 33 wt-%.

35. A method according to claim 1 further comprising pumping the liquid urine from the supply vessel through at least one prefilter before the liquid urine is pumped through the cartridge.

36. A method according to claim 35 wherein said at least one prefilter comprises a deep-bed filter or a precoated filter.

37. A method according to claim 35 wherein said at least one prefilter comprises a deep-bed filter having a sand bed, a filter cartridge, a plate filter, a cartridge filter, a filter bag, or a filtration tube.

38. A method according to claim 35 wherein a filtering aid is added to the liquid urine before filtration.

39. A method according to claim 1 wherein the step of storing the upright cartridge containing the solid adsorbent support with the concentrated and stabilized natural mixture of conjugated estrogens adsorbed thereon lasts for at least several days.

40. A method according to claim 39 wherein the step of storing lasts far at least one week.

41. A method according to claim 39 wherein the step of storing lasts from several days to several weeks.

42. A method according to claim 39 wherein the step of storing lasts from several days to eight weeks.

43. A method according to claim 39 wherein the step of storing lasts from one week to eight weeks.

44. A method according to claim 1 further comprising the step of processing said upright cartridge with a solid adsorbent support containing concentrated and stabilized pregnant mare urine.

45. A method according to claim 1 wherein the step of pumping a predetermined maximum total amount of collected liquid pregnant mare urine from a supply vessel through an upright cartridge containing a solid adsorbent support capable of adsorbing a predetermined quantity of conjugated estrogens takes place at or near a collection site of pregnant mare urine.

46. A method according to claim 1 wherein the processing site is remote from the collection site.

APPENDIX B

WHAT IS CLAIMED IS:

1. A method for obtaining a natural mixture, depleted in phenolic urine contents, of conjugated estrogens from pregnant mares' urine, comprising:

a) contacting a pregnant mare urine with an amount of a polymeric adsorption resin sufficient to absorb the mixture of conjugated estrogens contained in the urine, and separating a polymeric adsorption resin laden with the mixture of conjugated estrogens from the rest of the urine,

b) washing the polymeric adsorption resin laden with the mixture of conjugated estrogens with an alkaline washing water which has a pH of at least 12.0, and

c) contacting the washed adsorption resin with a sufficient amount of an elution liquid to desorb the mixture of conjugated estrogens adsorbed on the resin, said elution liquid comprising a water-miscible organic solvent selected from the group consisting of water-miscible ethers, lower alkanols and lower aliphatic ketones or a mixture of the water-miscible organic solvent and water which has optionally been rendered alkaline, and separating an eluate containing the natural mixture of conjugated estrogens from the adsorption resin, and optionally concentrating the separated eluate, wherein the polymeric adsorption resin laden with the mixture of conjugated estrogens is washed with water in an intermediate washing between process steps b) and c).

2. A method according to claim 1, wherein the urine in step a) is a urine freed of mucilaginous substances and solids, a reduced concentrate of such urine or a reduced urine retentate obtained by membrane filtration of such urine.

3. A method according to claim 1, wherein said intermediate washing is carried out at temperatures below room temperature.

4. A method according to claim 3, wherein said intermediate washing is carried out at a temperature between 0°C and 10°C.

5. A method according to claim 4, wherein said intermediate washing is carried out at a close to but above 0°C.

6. A method according to claim 1, wherein the washing water used for the intermediate washing operation is precooled to temperatures below room temperature.

7. A method according to claim 6, wherein the washing water used for the intermediate washing is precooled to a temperature between 0°C and 10°C.

8. A method according to claim 7, wherein the washing water used for the intermediate washing is precooled to a temperature close to but above 0°C.

9. A method according to claim 1, wherein the alkaline washing water used in step b) is precooled to a temperature below room temperature.

10. A method according to claim 9, wherein the alkaline washing water used in step b) is precooled to a temperature between 0°C and 10°C.

11. A method according to claim 10, wherein the alkaline washing water used in step b) is precooled to a temperature close to but above 0°C.

12. A method according to claim 1, wherein step b) and the intermediate washing are both carried out at temperatures below room temperature.

13. A method according to claim 12, wherein step b) and the intermediate washing are both carried out at temperatures between 0°C and 10°C.

14. A method according to claim 13, wherein step b) and the intermediate washing are both carried out at temperatures close to but above 0°C.

15. A method according to claim 1, wherein the polymeric adsorption resin is contained in a reactor, and the reactor containing the adsorption resin is precooled to a temperature below room temperature.

16. A method according to claim 15, wherein the reactor containing the adsorption resin is precooled to a temperature between 0°C and 10°C.

17. A method according to claim 16, wherein the reactor containing the adsorption resin is pre-cooled to a temperature close to but above 0°C.

18. A method according to claim 1, wherein the pregnant mare urine is pre-cooled to a temperature below room temperature.

19. A method according to claim 18, wherein the pregnant mare urine is pre-cooled to a temperature between 0°C and 10°C.

20. A method according to claim 19, wherein the pregnant mare urine is pre-cooled to a temperature close to but above 0°C.

21. A method according to claim 1, wherein the adsorption resin is a semipolar adsorption resin.

22. A method according to claim 21, wherein the adsorption resin is a non-ionic semipolar adsorption resin.

APPENDIX C

Amendments to the Claims:

The listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently Amended) A method for obtaining a natural mixture of conjugated equine estrogens depleted in non-conjugated lipophilic compounds selected from the group comprising non-conjugated flavonoids, non-conjugated isoflavonoids and non-conjugated norisoprenoids , said method comprising:

a) subjecting an aqueous initial phase, selected from the group consisting of

(i) an aqueous solution of a natural mixture of conjugated estrogens from pregnant mares' urine already depleted in phenolic urine contents comprising phenol, cresol and dihydro-3,4-bis[(3-hydroxyphenyl)methyl]-2(3H)-furanone and obtained by solid-phase or liquid-liquid extraction ;

(ii) an aqueous concentrate of a natural mixture of conjugated estrogens from pregnant mares' urine already depleted in phenolic urine contents comprising phenol, cresol and dihydro-3,4-bis[(3-hydroxyphenyl)methyl]-2(3H)-furanone and obtained by solid-phase or liquid-liquid extraction ;

(iii) a concentrate of a urine liquid, and

(iv) a urine concentrate, freed from mucilaginous substances and solids by mechanical separation methods, or a concentrated urine retentate obtained by filtration ~~optionally pre-purified by filtration~~ ,

to a liquid-liquid extraction with a sufficient quantity of an organic solvent extracting agent which is suitable for extracting non-conjugated lipophilic compounds and is at most only slightly miscible with water, and subsequently separating a resulting aqueous phase , whereby the organic solvent extracting agent is selected from the group consisting of straight-chained, branched or cyclic C_4-C_{10} alcohols, C_2-C_{10} esterified acids, C_3-C_{10} aldehydes, C_4-C_{10} ketones, C_2-C_{10} ethers, C_3-C_6 nitriles and C_1-C_3 haloalkanes and mixtures of the aforementioned solvents ;

b) optionally repeating method step (a) with the resulting aqueous phase, and

c) recovering an aqueous phase containing the natural mixture of conjugated estrogens depleted in non-conjugated lipophilic compounds selected from the group comprising non-conjugated flavonoids, non-conjugated isoflavonoids and non-conjugated norisoprenoids .

2. (Original) A method according to claim 1, further comprising concentrating the recovered aqueous phase containing the natural mixture of conjugated estrogens.

3. (Cancelled)

4. (Cancelled)

5. (Original) A method according to claim 1, wherein said extracting agent is immiscible with water.

6. (Currently amended) A method according to claim 1, wherein the aqueous initial phase in method step a) comprises (i) an aqueous solution of a natural mixture of conjugated estrogens from pregnant mares' urine already depleted in phenolic urine contents, or (ii) an aqueous concentrate of a natural mixture of conjugated estrogens from pregnant mares' urine already depleted in phenolic urine contents.

7. (Cancelled)

8. (Currently Amended) A method according to claim [[7]] 1, wherein the extracting agent is selected from the group consisting of C_1-C_4-alkyl acetates, hexanol, diethyl ether, methylene chloride, methyl tert.-butyl ether and mixtures of two or more of the aforementioned solvents.

9. (Original) A method according to claim 8, wherein the extracting agent comprises a C_1-C_4-alkyl acetate.

10. (Original) A method according to claim 9, wherein the extracting agent is ethyl acetate.

11. (Original) A method according to claim 1, wherein in method step (a) the aqueous initial phase is adjusted to a pH value in the range from 4 to 12.

12. (Original) A method according to claim 11, wherein the pH value in step (a) is in the range from 4.0 to 7.0.

13. (Original) A method according to claim 12, wherein the pH value in step (a) is in the range from 4.0 to 6.0.

14. (Original) A method according to claim 13, wherein the pH value in step (a) is in the range from 4.7 to 5.3.

15. (Original) A method according to claim 1, wherein in method step (a) the volume ratio of the aqueous initial phase to the extracting agent lies in the range from 5:1 to 1:3.

16. (Original) A method according to claim 15, wherein the volume ratio of the aqueous initial phase to the extracting agent lies in the range from 2:1 to 1:2.

17. (Original) A method according to claim 1, wherein in method step (b) the volume ratio of the aqueous initial phase obtained from step (a) to the extracting agent lies in the range from 20:1 to 1:1.

18. (Original) A method according to claim 17, wherein in step (b) the volume ratio of the aqueous initial phase obtained from step (a) to the extracting agent lies in the range from 10:1 to 2:1.

19. (New) A method according to claim 6, wherein the depletion in phenolic urine contents is obtained by solid-phase extraction on a non-ionic semipolar adsorption resin or on a hydrophobized silica gel.

APPENDIX D

WHAT IS CLAIMED IS:

1. A method for obtaining a natural mixture of conjugated estrogens from pregnant mares' urine, comprising:

- contacting a urine with an amount of a polymeric adsorption resin sufficient to adsorb the mixture of conjugated estrogens contained in the urine, and separating a resulting polymeric adsorption resin loaded with the mixture of conjugated estrogens from the rest of the urine;
- washing the polymeric adsorption resin loaded with the mixture of conjugated estrogens with a washing water which has been adjusted to a pH value of at least 12.0;
- contacting the washed adsorption resin with a sufficient amount of an elution liquid to desorb the mixture of conjugated estrogens loaded thereon, and
- separating an eluate containing the natural mixture of conjugated estrogens from the adsorption resin;

wherein:

- the natural mixture of conjugated equine estrogens is depleted in phenolic urine contents and in non-conjugated lipophilic compounds, and comparable non-conjugated compounds, and
- the separated eluate is a one-phase or two-phase mixture containing:
    (iii) water, and
    (iv) at least one water-immiscible or only slightly water-miscible organic solvent suitable for the elution of non-conjugated lipophilic compounds; and
- if the separated eluate is a two-phase mixture, further comprising separating the phases and recovering an aqueous phase containing the natural mixture of conjugated estrogens.

2. A method according to claim 1, wherein the starting urine is a urine freed of mucilaginous substances and solids, a reduced concentrate of

such a urine, or a reduced urine retentate obtained by membrane filtration of such a urine.

3. A method according to claim 1, wherein the polymeric adsorption resin loaded with the mixture of conjugated estrogens subjected to an intermediate washing with water between the washing with water which has been adjusted to a pH of at least 12.0 and the contacting with the elution liquid.

4. A method according to claim 1, wherein the recovered natural mixture of conjugated equine estrogens is depleted in androstane and pregnane steroids.

5. A method according to claim 1, wherein the elution liquid further comprises at least one water-miscible organic solvent selected from the group consisting of water-miscible ethers, lower alkanols and lower aliphatic ketones.

6. A method according to claim 1, wherein the water in the elution liquid is adjusted to a pH value in the alkaline range.

7. A method according to claim, further comprising reducing the volume of the separated eluate.

8. A method according to claim 1, wherein said non-conjugated lipophilic compounds are selected from the group consisting of non-conjugated flavonoids, non-conjugated isoflavonoids, non-conjugated norisoprenoids, and non-conjugated steroids.

9. A method according to Claim 1, wherein the elution liquid is a one-phase or two-phase mixture comprising:

(i) water;

(ii) at least one water-immiscible or slightly water-miscible organic solvent suitable for eluting non-conjugated lipophilic compounds selected from the group consisting of non-conjugated flavonoids, non-conjugated isoflavonoids, non-conjugated norisoprenoids, and non-conjugated steroids; and

(iii) at least one water-miscible organic solvent selected from the group consisting of water-miscible ethers, lower alkanols, lower aliphatic ketones, and mixtures of two or more of the foregoing.

10. A method according to claim 9, wherein the water in the elution liquid is adjusted to an alkaline pH value.

11. A method according to claim 1, wherein the organic solvent in (ii) is selected from the group consisting of straight-chain, branched or cyclic C_4-C_{10} alcohols, C_1-C_{10} esterified acids, C_3-C_{10} aldehydes, C_4-C_{10} ketones, C_3-C_{10} ethers, C_3-C_6 nitriles, C_1-C_3 haloalkanes, and mixtures of two or more of the foregoing.

12. A method according to claim 11, wherein the organic solvent in (ii) is selected from the group consisting of C_1-C_4-alkyl acetates, butanol, cyclohexanol, hexanol, diethyl ether, methylene chloride, methyl tert. butyl ether, and mixtures of two or more of the forgoing.

13. A method according to claim 12, wherein the organic solvent in (ii) is selected from the group consisting of C_1-C_4-alkyl acetates and mixtures thereof.

14. A method according to claim 13, wherein the organic solvent in (ii) is ethyl acetate, isopropyl acetate, or a mixture thereof.